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Photoreceptor or statocyst ? The ultrastructure and function of a unique sensory organ embedded in the shell valves of *Cryptoplax mystica* Iredale & Hull, 1925 (Mollusca:Polyplacophora)

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ABSTRACT

Cryptoplax mystica is a common inhabitant of the New South Wales mid-north coast. An elongate chiton with reduced shell valves, it is usually found in narrow crevices and holes in sand-sponge aggregates in the lower intertidal zone. A recent examination of some head, intermediate and tail valves revealed a unique 'sense organ' imbedded in pigmented tissue immediately below the shells dorsal surface. The structure was found to possess features consistent with a photoreceptive and/or balance function, and to display a degree of complexity hitherto undescribed for the Polyplacophora.

INTRODUCTION

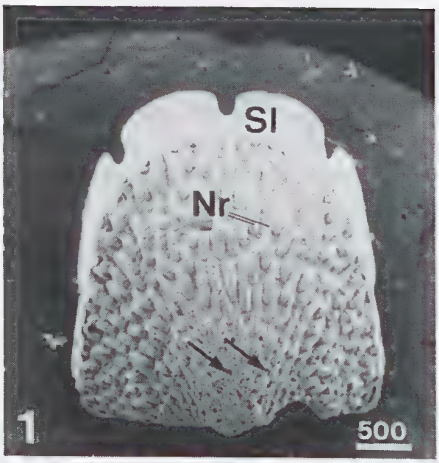
A number of studies have been conducted on the ultrastructure of aesthetes in chitons during recent years (Boyle, 1974; Haas and Kriesten, 1978; Fischer, 1978b, 1979, 1988; Fischer and Renner, 1978; and Baxter et al., 1987). This comparative research has identified both sensory and secretory organelles as consistent features of aesthetes, in the small range of species examined to date, and has provided valuable information on the potential function/s of these complex structures. Most researchers now contend, on the basis of fine-structural detail, that aesthetes function as photoreceptors and/or periostracum secreting organs.

Although aesthetes and their associated micraesthete ducts are the numerically dominant structure to be found embedded within the shell valves of chitons, some species possess supplementary organs e.g. photoreceptive ocelli (Moseley, 1885; Boyle, 1969; Currie, 1989). This paper investigates the discovery of a new class of organ found in the shell valves of *Cryptoplax mystica* Iredale & Hull, 1925. Detailed observations on the gross structure of this new 'pigmented' cavity, and it's relationship to the various shell valve layers are presented. In addition, the fine-structural detail of the enclosed soft tissues are examined.

MATERIALS AND METHODS

Specimens of *C. mystica* were collected from rocks in the intertidal region at Arrawarra on the mid-north coast of New South Wales (30° 10'S, 153° 20'E). These animals were kept in aerated sea-water for no longer than one hour prior to the removal of three principal shell valves (i.e. one head, intermediate, and tail valve).

The internal arrangement of the pigmented channels and their relationship to the shell valve layers was investigated by examination of surfaces exposed by fractures.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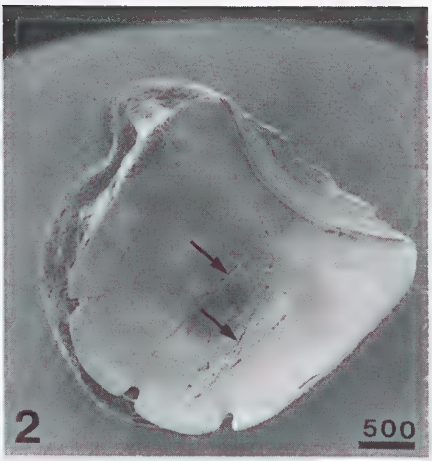
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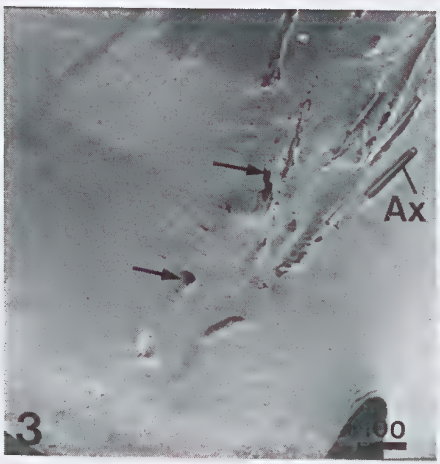
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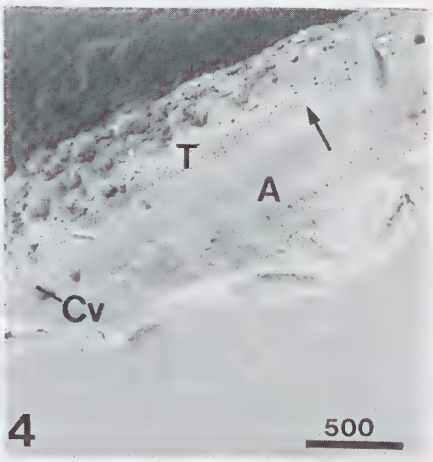
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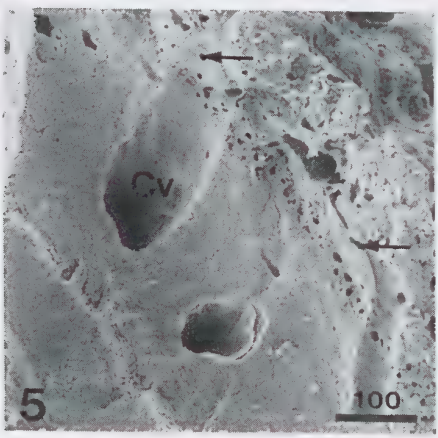
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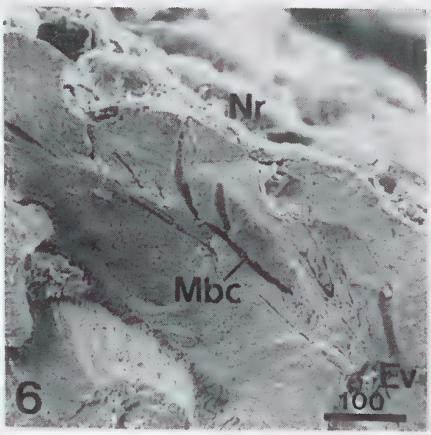
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After washing in distilled water, valves were ultrasonically cleaned and dehydrated in an alcohol series. Pieces of valve were subsequently mounted with colloidal copper on aluminium stubs, sputter-coated with gold, and the fractured surfaces examined at an accelerating voltage of 20 kV on a JEOL JSM35 scanning electron microscope.

Material for transmission electron microscopy was fixed in 3% glutaraldehyde in phosphate buffer pH 7.2 at room temperature for 2 hours, rinsed in cold 0.2M phosphate buffer every hour for 5 hours, then decalcified in chilled 5% disodium ethylenediaminetetraacetic acid (EDTA) in phosphate buffer. The pieces of shell were post-fixed at room temperature for one hour in 2% osmium tetroxide, dehydrated in increasing concentrations of alcohol, then infiltrated and embedded in Spurr low-viscosity medium. Sections were cut on a Reichart ultramicrotome, mounted on copper grids, and then stained with saturated uranyl acetate and lead citrate (Reynolds, 1963), before being examined on a JEOL 1200EX electron microscope at an accelerating voltage of 60kV.

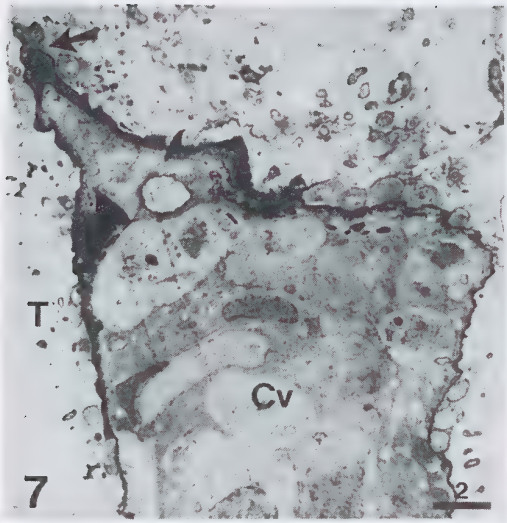
Semi-thin sections of shell were cut from blocks prepared as above for transmission electron microscopy. These were mounted on glass slides and stained with 4% toluidine blue, before being examined on an Olympus CH light microscope.

The valve nomenclature used in this study is essentially that defined by Baxter and Jones (1984), unless otherwise indicated.

RESULTS

Pigmented cavities occur in association with aesthete channels in the mid-posterior tegmentum of anterior valves, and throughout the jugal regions of the intermediate and posterior valves. They occur most frequently on the anterior valve, with up to 30 structures being identified in single specimen. Unlike aesthete systems, the pigmented cavities do not terminate at the shell surface in a circular apical cap, and hence are only visible in individuals where the dorsal surface has been eroded (Fig.

◀ **Figures 1-6.** 1. Scanning electron micrograph of the dorsal surface of an anterior valve of *Cryptoplax mystica*, showing nodulose ridges (Nr) radiating from the mid/posterior region towards the sutural lamina (Sl). Openings visible in the mid/posterior area (arrowed) are associated with pigmented cavities that extend through the shell valve layers to the ventral surface. 2. Ventral surface of an anterior valve of *Cryptoplax mystica* showing slits in the hypostracum (arrowed) representing the proximal openings of pigmented cavities. 3. Proximal openings of pigmented cavities (arrowed) on the ventral surface of an anterior valve of *Cryptoplax mystica*. Note the presence of a single axon fibre (Ax) running through the lower region of each cavity. 4. Transverse fracture through an anterior valve of *Cryptoplax mystica* showing pigmented cavities (Cv) extending through the tegmentum (T) / articulamentum (A) interface (arrowed). Note that the small perforations in the tegmentum, which represent multiple-branch aesthete channels, do not penetrate the articulamentum — the aesthete structures have been observed to extend obliquely from the dorsal surface to the eave tissue. 5. Transverse fracture through an anterior valve of *Cryptoplax mystica* showing aesthete channels (arrowed) opening into the distal region of a pigmented cavity (Cv). 6. Transverse fracture through an anterior valve of *Cryptoplax mystica* showing multiple branch aesthete channels (Mbc) extending from the nodulose ridges (Nr) on the dorsal surface to the eave tissue (Ev). Scale bars in micrometers.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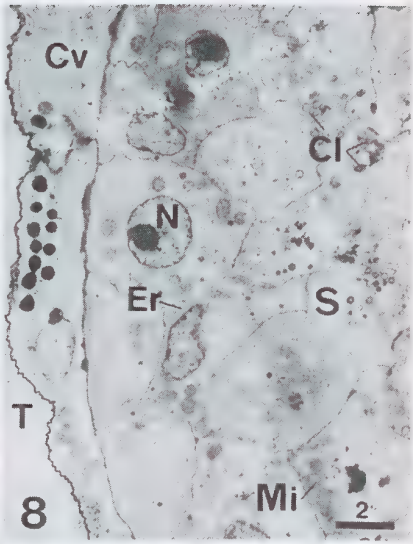
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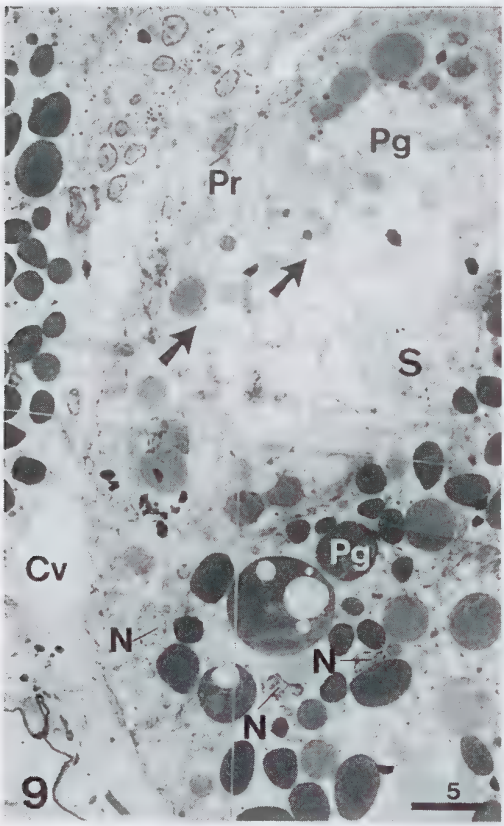
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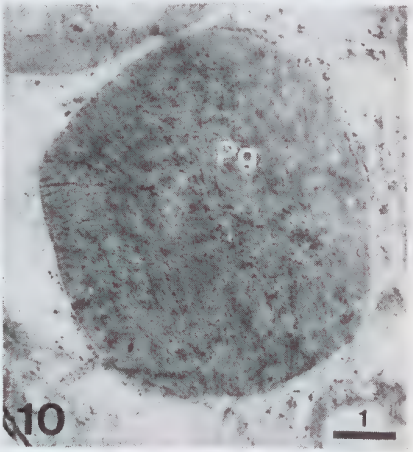
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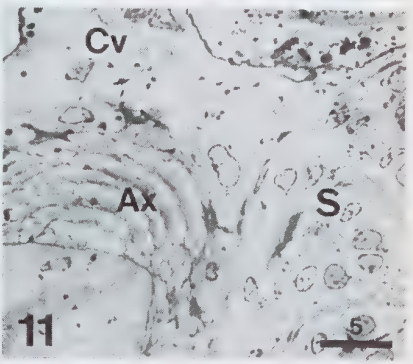
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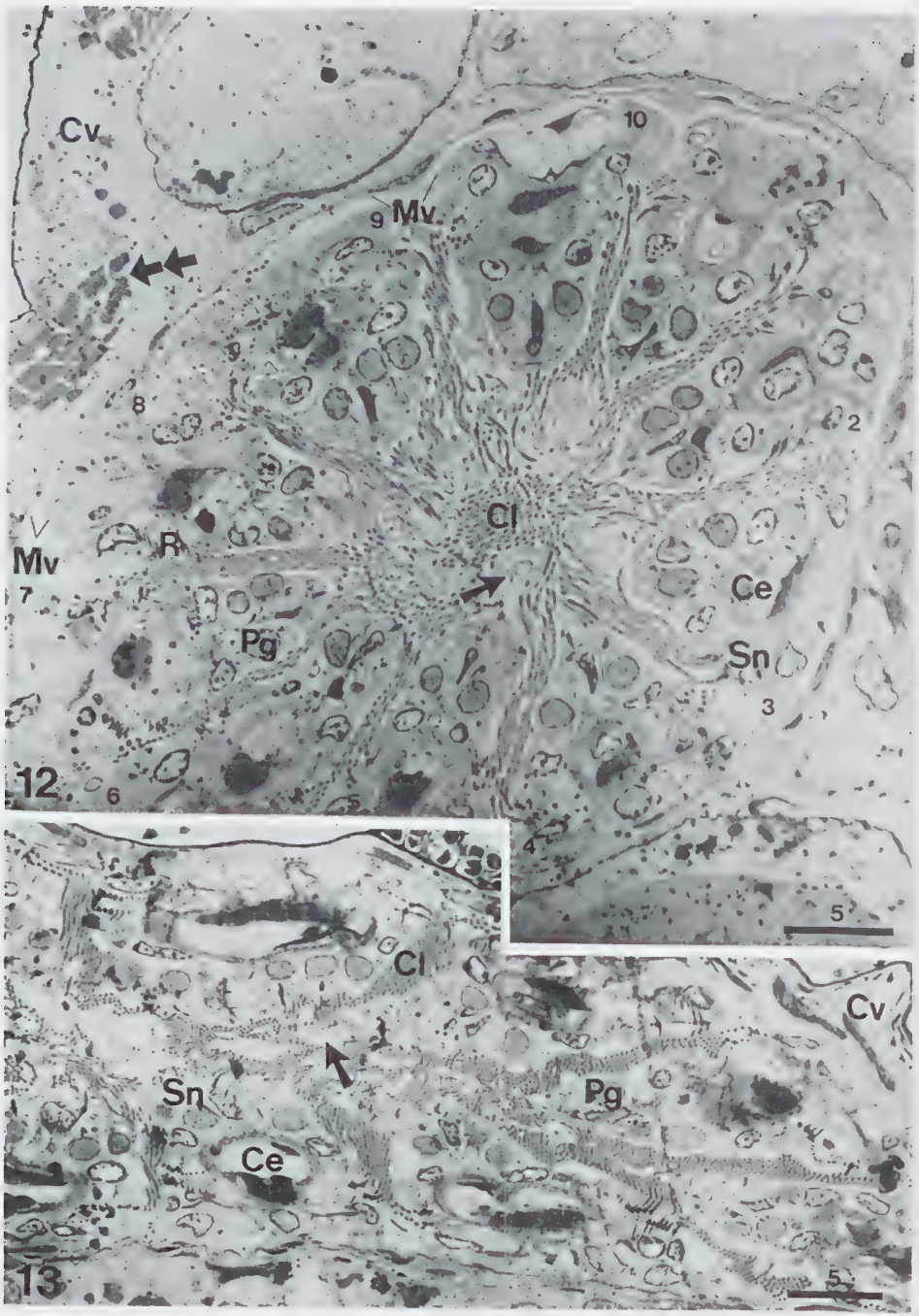
1). From the dorsum, the cavities extend obliquely through the shell layers, becoming progressively narrower towards the anterior of the valve. They eventually terminate at the ventral surface in narrow slits (Fig. 2) or ovoid apertures. It is apparent that the soft tissues enclosed by the pigmented cavity continue to extend towards the mantle, as axon fibres are often observed protruding from the ventral surface (Fig. 3).

A transverse fracture through an anterior shell valve (Fig. 4) confirms that the pigmented cavities extend across the tegmentum / articulamentum interface, whilst multiple-branch aesthetes are restricted to the upper shell layer. The aesthetes are, however, closely associated with the pigmented organs, and numerous aesthete and micraesthete channels open into the distal region of the cavity (Fig. 5). The cavities are clearly much larger than the surrounding aesthete channels (Fig. 6), and indeed any other aesthete previously described, being up to 150 μm in width and 300 μm in length.

The cavity is separated from the shell surface by a thin layer (approximately 2 μm) of unperforated periostracum-like material, and the enclosed distal region appears to be packed with a disorganized array of cells with variable contents (Fig. 7). A single spherical 'sensory' organ (approximately 60 μm in diameter) is contained in the lumen of the cavity ventral to this. In certain sections, the sense organ (Fig. 8) appears to consist of a collection of cells reminiscent of the retinal cell bodies found in the ocelli of *Acanthopleura gaimardi* (De Blainville, 1825) (Currie, 1990); each cell comprises a rounded nucleus showing little condensed chromatin, finely granular cytoplasm rich in mitochondria and endoplasmic reticulum, and an association of ciliary profiles. Other sections through the same organ (Fig. 9) reveal a cloven organization; with each segment composed of a group of cells packed with pigment granules, and separated from adjacent cell groups by narrow fissures running between the centre and circumference of the structure. The rounded

◀ **Figures 7-11.** 7. Longitudinal section through part of the distal region of a pigmented cavity (Cv) within the tegmentum (T) of a shell valve of *Cryptoplax mystica* showing the thin layer of periostracum-like material (arrowed) which covers an irregular association of cells at the shell surface. **8.** Section through the lumen of a pigmented cavity (Cv) found within the tegmentum (T) of a shell valve of *Cryptoplax mystica* showing a peripheral portion of a specialised sense organ (S). The organ here is comprised of uniform cells which extend throughout the entire circular profile. Each of these cells contains a rounded nucleus (N) with little condensed chromatin, many mitochondria (Mi), endoplasmic reticulum (Er), and an association of cilia (Cl). **9.** Section through the lumen of a pigmented cavity (Cv) in *Cryptoplax mystica* showing part of the circular profile of a sensory organ (S). Here, cells packed with pigment granules (Pg) form groups, as evidenced by the close association of nuclei (N), which appear to be separated from adjacent groups by narrow membranous fissures (arrowed) running between the centre and circumference of the organ. A narrow layer of peripheral (Pr) cells, absent of pigment granules, extends throughout much of the inner surface of the organ wall. **10.** Section through a pigment granule found within the specialized sensory organ of *Cryptoplax mystica* showing an internal organization of fine lamellae. **11.** Section through a pigmented cavity (Cv) in *Cryptoplax mystica* showing a structure resembling a collection of axons (Ax) adjacent to a ciliated portion of the sensory cell (S). Scale bars in micrometers.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pigment granules found here are distinctly variable in size (0.5 - 8 μm), density (opaque to electron-dense), and structure (finely granular to lamellate (Fig. 10)).

Perhaps the most curious feature of the entire structure is the array of ciliary processes, evident in the proximal region adjacent to a component resembling an afferent neuron (Fig. 11). Transverse and oblique sections through this area (Figs. 12 and 13 respectively) display a unique arrangement of ten evenly spaced cell groups placed around the inner wall of the organ, each of which give rise to, and is separated by, bundles of inwardly directed elongate cilia. The cell groups themselves are composed of seven or more uniform 'sensory' cells which surround an elongate central cell: each sensory cell contains a small rounded nucleus and a few mitochondria within the finely granular cytoplasm, and gives rise, peripherally, to a row of eight to ten cilia (9 + 2) up to 15 μm in length (Fig. 14); the central cells do not appear to possess basal bodies, and consist of two distinct regions 1) an epicentric electron-dense region, and 2) a centrally orientated electron-light region containing the nucleus. Each of the ten sensory cell groups is individually bound by a membrane bearing short microvillous-like projections on all surfaces. A similar membrane covers the entire inner surface of the organ wall, which is comprised of a small number of squamous peripheral cells. Other notable features evident in these later sections through the organ are the vitreous plate-like structures (perhaps decalcified statoliths) found within the central ciliated region (Figs. 12 - 14), and the occurrence of electron-dense rhombic structures (possibly crystalline reflectors) situated in the pigmented cavity, between the sensory organ and the shell tegmentum (Fig. 12).

A hypothetical arrangement of the major cellular features within the sensory organ, based on semi-thin and transmission electron micrographs, is given in Fig. 15.

◀ **Figures 12-13.** **12.** Transverse section through the proximal region of a sensory organ within the lumen of a pigmented cavity (Cv) in *Cryptoplax mystica* showing the peripheral arrangement of sensory cell cell groups (1 - 10) giving rise to inwardly directed bundles of cilia (Cl). Each of the ten cell groups consist of a single central cell (Ce), surrounded by a number of sensory cells (Sn); the former displays a epicentric electron-dense region and an inner electron-light region containing the nucleus, while the latter cell type contains small pigment granules (Pg) and rows of ciliary roots (R). The cell groups are covered marginally by a continuous membrane bearing short microvillous projections (Mv) at regular intervals. A similar membrane extends to cover the entire inner surface of the organ wall. Small vitreous structures (single arrow) found in the central ciliated region of the organ may represent decalcified statoliths. The electron-dense rhombic structures (double arrow) within the pigmented cavity are reminiscent of the crystalline light reflectors found in some bivalves. Such crystals were a regular feature of several different sections, and therefore, cannot be artifacts. **13.** Oblique section through the proximal region of a sensory organ within the lumen of a pigmented cavity (Cv) in *Cryptoplax mystica* showing numerous sense cells (Sn) with pigment granules (Pg) surrounding the elongate central cells (Ce) and giving rise to bundles of ciliary processes. Vitreous interruptions in the cilia bundles (arrowed) may represent decalcified statoliths. Scale bars in micrometers.

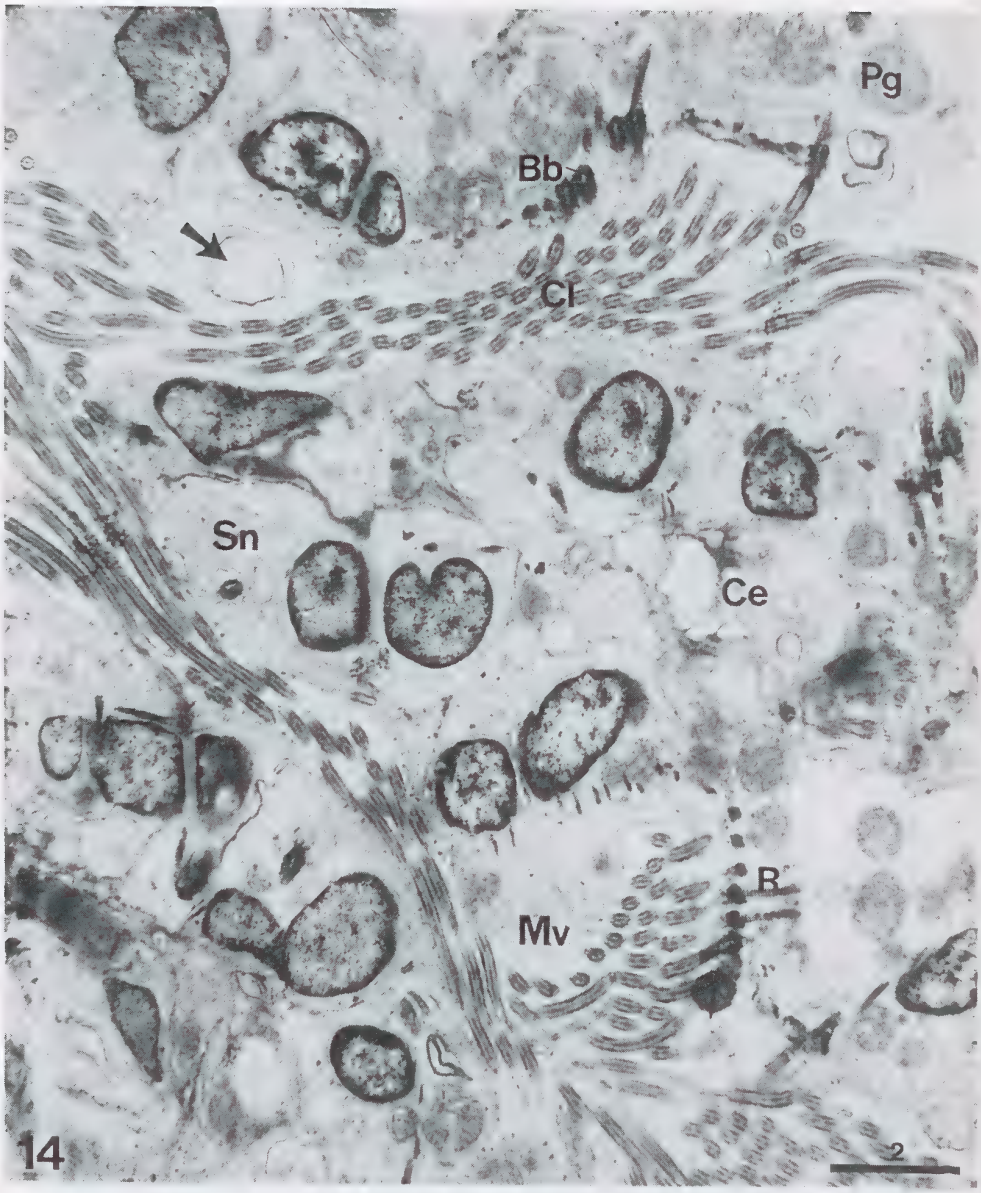

Figure 14. Transverse section through a sensory cell group within the peripheral region of sense organ in *Cryptoplax mystica* showing the arrangement of ciliary roots (R) and basal bodies (Bb) associated with each sense cell (Sn). A number of lamellate pigmented granules (Pg) may, in addition, be present in the sense cells which encircle the single central cell (Ce). Each sensory cell group is covered marginally by a continuous membrane bearing short microvillous projections (Mv), and is separated from adjacent cell groups by fissures containing bundles of cilia (Cl) emanating from perimetric sense cells. The opaque structure in the lumen of the sensory organ (arrowed) may represent a decalcified statolith. Scale bar in micrometers.

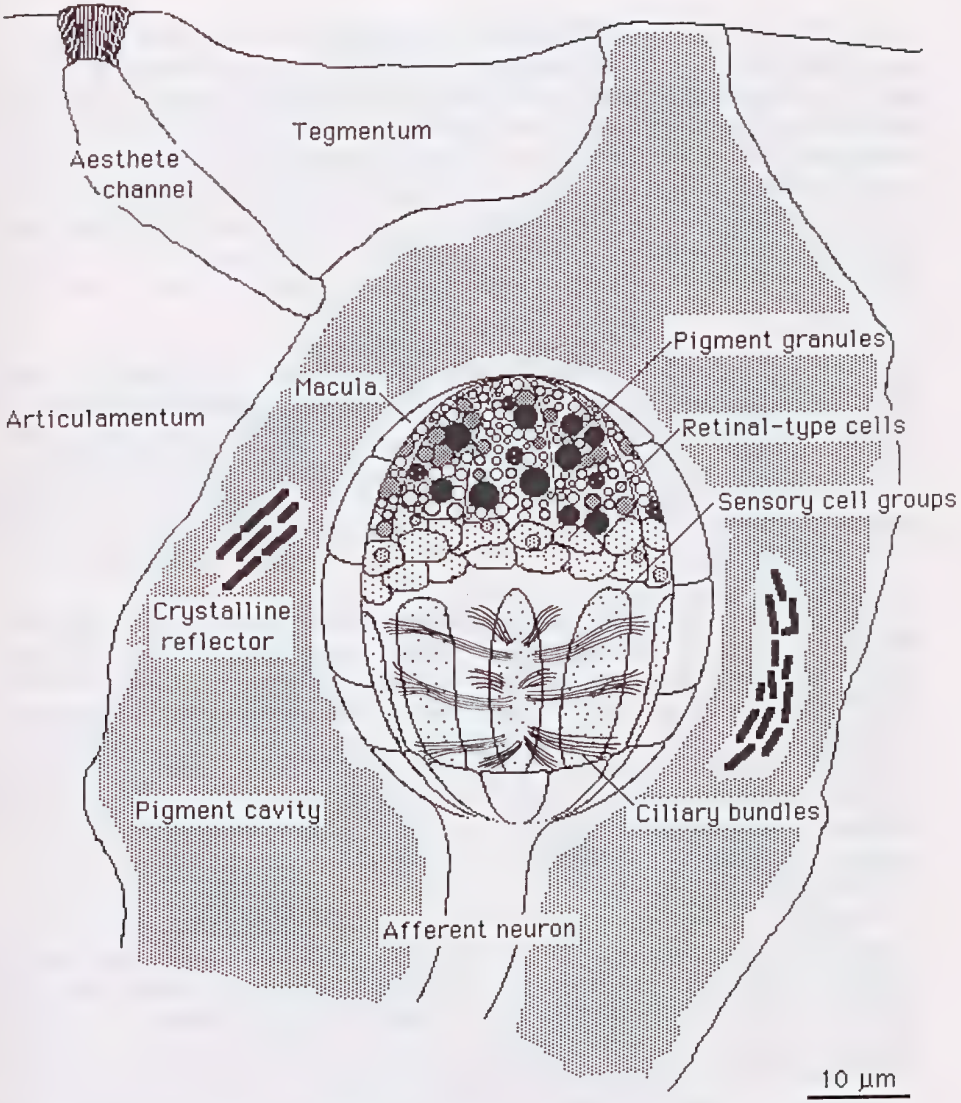

Figure 15. Hypothetical three-dimensional reconstruction of the specialised sense organ found within the pigmented cavities of *Cryptoplax mystica*.

DISCUSSION

Intrapigmental ocelli of quite different construction have been described in a variety of chiton sub-families, ranging from the less advanced Callochitoninae (Ischnochitonidae), to the phylogenetically 'higher' Toniciinae and Acanthopleurinae (Chitonidae) (Nowikoff, 1907, 1909). In *Acanthopleura*, a small convex lens embedded in the pigmented region of the tegmentum is described by

Nowikoff as being surrounded by a fibrillar area (possibly bundles of cilia), a cup of retinal cells, and a layer of pigment. But for the absence of a lens, the sensory organ of *C. mystica* displays all of these features indicative of light reception. Further support for this assumption is found in a comparison with the ultrastructure of the pallial eyes of the bivalve *Pecten maximus* (L.) (Barber et al., 1967). This species possesses both ciliated primary photoreceptor cells (similar to the ciliated sensory cells of *C. mystica*), and secondary cells bearing short microvilli (analogous with the microvillous membrane). In addition, the eye of *P. maximus* is surrounded by a series of argentea (crystalline reflectors), which closely resemble those found in *C. mystica*.

If indeed the sensory organ of *C. mystica* functions as a photoreceptor, the dorsal pigmented region probably acts as a spectral filter; the segmentation of the pigment cell groups and the variance in granule size and density might allow a highly selective penetration of light stimuli to specific regions of the microvillous membranes below. Such a system could be expected to differentiate light intensity as well as direction (i.e. those criteria relating to photoperiod and orientation). Changes in reflected light from the argentea might, in addition, directly stimulate the array of ciliary receptors, and facilitate a shadow response similar to that described for the scallop *P. maximus*.

The evolution and functional significance of photoreceptor design is unclear (Westfall, 1982). The occurrence of ciliary type receptor cells within the pigmented regions of the shell valves of *C. mystica* is particularly interesting, not only because this type of receptor generally occurs in deuterostomes, but because this species represents the most highly specialised family of all Polyplacophora (according to the current classification system of Kass and Van Belle, 1980), the Cryptoplacidae. Rudimentary ciliary type membranes have been described in the extrapigmental ocelli of *Onithochiton neglectus* De Rochebrune, 1881 (Boyle, 1969) and *A. gaimardi* (Currie, 1990), and may prove to be the basis for the development of this specialised organ.

Similarities in the peripheral arrangement of the ciliated sensory cells of *C. mystica* with geotactic sense organs in other invertebrates, the linear organisation of ciliary roots within the sense cells, and the occurrence of vitreous plate-like structures in the lumen, all suggest an additional/alternative function, i.e. that of a statocyst. Such structures have been described in all molluscan classes with the exception of Polyplacophora (Barber, 1968), and have been shown to display an orientation function in both Gastropods (Lever and Geuze, 1965) and Lamellibranchs (Buddenbrock, 1915). A statocyst could be of considerable benefit to *C. mystica* in respect of its habitat; the animal is normally found wedged in small crevices under boulders, and rarely ventures from this abode to a position in which light might influence its orientation (phototaxis). A perception of gravity, and hence an ability to orientate in such a tenebrous environment, could be facilitated by a statocyst. However, until suitable methodologies are developed to experimentally manipulate these sensory organs and measure responses, like the aesthete — one can merely speculate on function.

ACKNOWLEDGEMENTS

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